PENAPISAN AKTIVITAS ANTIPROTOZOA DALAM BIJI SAGA (*Adenanthera pavonina* LINN)

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INTISARI

*Protozoa **Tetrahymena pyriformis** GL adalah suatu eukaryot, yang metabolismenya menyerupai mammalia, sehingga banyak dipergunakan sebagai mikroorganisma penguji dalam penentuan kualitas protein juga dalam uji toksisitas. Dalam rangka penelusuran sifat toksis atau antinutrisi dalam biji saga (**Adenanthera pavonina** LINN) sifat antiprotozoa dari ekstrak-ekstrak biji saga yang diperoleh dari hasil ekstraksi berturut-turut dengan n-heksana dan etanol, telah diuji. Dari hasil pengamatan populasi sel total dan sel hidup protozoa, didapatkan bahwa ekstrak minyak saga pada kadar sampai 0,1 % dalam medium pertumbuhan tidak menunjukkan pengaruh yang nyata terhadap pertumbuhan **T. pyriformis** GL selama 96 jam inkubasi pada 30°C. Sedangkan ekstrak etanol saga menunjukkan hambatan yang nyata terhadap pertumbuhan **T. pyriformis** GL mulai 7 jam inkubasi pada 30°C. Kadar 0,1% ekstrak etanol saga dalam medium menghambat pertumbuhan **T. pyriformis** GL sebanyak 55,1% dan 1% ekstrak etanol menunjukkan hambatan sebanyak 87,6% (pada 24 jam inkubasi). Ekstrak etanol saga menunjukkan sifat antiprotozoa yang paling besar dibandingkan ekstrak lainnya. Analisis kualitatif ekstrak etanol saga memberikan hasil positif terhadap saponin dan alkaloida dan hasil analisis dengan kromatografi cair kinerja tinggi menunjukkan adanya paling sedikit delapan komponen dalam ekstrak etanol saga.*

ABSTRACT

*The protozoa **Tetrahymena pyriformis** GL is an eucaryote. Its metabolism is similar to that of mammalia, so that it is widely used as a biological tool in protein quality as well as toxicity assays. In the framework of searching toxic or antinutritive properties in saga seed (**Adenanthera pavonina** LINN), extracts of saga bean were tested for their antiprotozoa activity. The saga bean extracts were obtained after successive extraction with n-hexane and ethanol. Observation of total and motile cell population indicated that 0,1% saga oil in the medium did not show significant effect on the growth of **T.pyriformis** GL during 96 hours incubation at 30°C. While ethanol extract of saga showed significant inhibition on the growth of **T.pyriformis** GL. Smaller cell population was already observed at 7 hours incubation at 30°C. At 24 hours incubation, 0,1% and 1% ethanol extract of saga in the medium showed 55,1% and 87,6% inhibition respectively. Ethanol extract showed the largest antiprotozoal activity compared to the other extract. Qualitative analysis indicated the presence of saponin and alkaloid in the ethanol extract of saga bean. Chromatographic analysis with high performance liquid chromatography showed the presence of at least eight components in the ethanol extract of saga.*

PENDAHULUAN

Tetrahymena pyriformis termasuk phylum protozoa, berbentuk lonjong dengan ukuran rata-rata 50x30μ. Mudah tumbuh dalam perbenihan bebas bakteri atau pun dalam perbenihan sintetis. *T.pyriformis* merupakan eukaryot, organel-organel dan membran dinding sel sama dengan sel eukaryot. Kebutuhan nutrisi dan metabolisma dalam tubuh *T.pyriformis* menyerupai metabolisma mamalia (hewan tinggi), karena itu protozoa ini banyak dipergunakan sebagai organisma penguji (1). Dibandingkan dengan penggunaan mammalia seperti tikus, uji toksisitas menggunakan protozoa dapat lebih cepat dan contoh yang diperlukan lebih sedikit. Hasil penelitian Otsuka *et al.* (2) menunjukkan bahwa urutan daya toksisitas beberapa fungisida terhadap *T.pyriformis* adalah sama dengan terhadap tikus, sehingga toksisitas terhadap *T.pyriformis* dapat digunakan sebagai indeks toksisitas terhadap mammalia.

T.pyriformis telah digunakan sebagai organisma penguji dalam penelitian toksisitas a-tomatine (3), senyawa kimia dalam air limbah (4,5,6,7), fungisida (2), mikotoksin (8), insektisida (9), senyawa senyawa hasil fermentasi (10) dan penentuan aktivitas biologi rubratoksin A dan B (11). Penelitian-penelitian tersebut menggunakan *T.pyriformis* untuk menguji toksisitas, dengan mengamati populasi sel, transmitans/absorbans atau kecepatan pemakaian oksigen (*respiratory response*). Mojzis et al (12) telah meneliti pengaruh senyawa-senyawa dichlorvos dan polichlorinated biphenyls terhadap aktivitas enzim esterase, transferase, dehidrogenase dan fosfatase dalam *Tetrahymena pyriformis*. Phillipson et al (13) melaporkan bahwa banyak tumbuh-tumbuhan tropis merupakan sumber senyawa-senyawa antiprotozoa (13).

Dalam penelitian ini *T.pyriformis* digunakan sebagai mikroorganisma penguji dalam penapisan sifat antiprotozoa biji saga. Seperti diketahui biji saga (*Adenanthera pavonina* LINN) komposisi kimianya seperti kedelai, mempunyai potensi sebagai sumber protein nabati (14). Akan tetapi penelitian pendahuluan terhadap tikus menunjukkan bahwa biji saga mempunyai sifat antinutrisi, dimana didapatkan

hambatan pertumbuhan tikus (15). Sehubungan dengan ini akan ditelusuri sifat toksis atau antinutrisi biji saga, dengan menguji aktivitas ekstrak-ekstrak biji saga terhadap *T.pyriformis*.

BAHAN DAN METODA

Biji saga

Biji saga diperoleh dari Juana, Pati, Jawa Tengah.

Organisma

Organisma yang dipergunakan ialah *Tetrahymena pyriformis* GL, yang berasal dari Macquarie University, Australia.

Bahan kimia

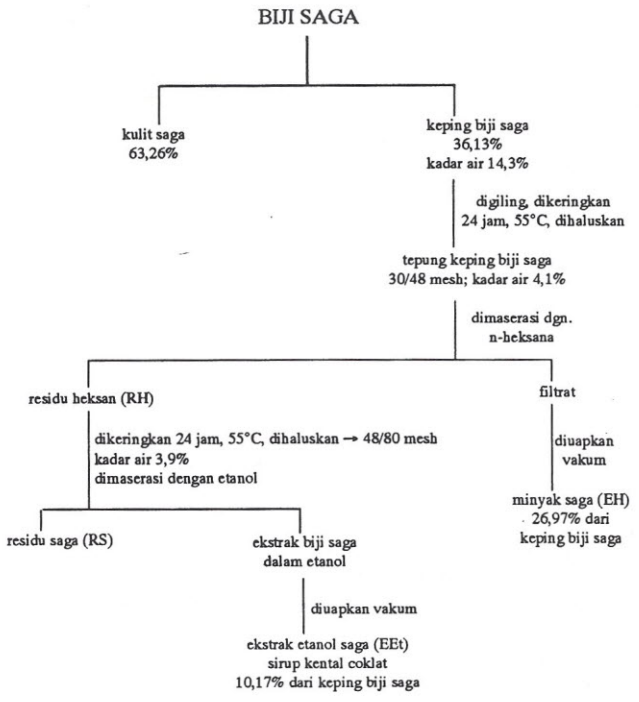
Bahan-bahan kimia dengan mutu pro analisis dari E. Merck, bahan medium dari Difco, pelarut organik kualitas teknis yang didestilasi ulang, digunakan dalam penelitian ini.

Pengolahan biji saga

Biji saga berkulit merah, keras dan tebal. Pemecahan biji saga dilakukan dengan mesin penggiling sehingga terbelah menjadi dua bagian. Keping biji saga dipisahkan dari kulit secara manual, kemudian dihaluskan sehingga diperoleh tepung saga dengan ukuran 30/48 mesh.

Ekstraksi biji saga

Keping biji saga diekstraksi dengan cara maserasi, berturut-turut dengan n-heksana dan etanol, seperti terlihat pada bagan 1. Pelarut diuapkan pada alat penguap berputar sehingga diperoleh ekstrak heksana (minyak saga; EH) dan ekstrak etanol saga (EEt) disamping residu heksana (RH) dan residu saga (RS).



Bagan 1. Ekstraksi keping biji saga dengan n-heksana dan etanol

Kurva pertumbuhan *T.pyriformis* GL.

Protozoa *T.pyriformis* GL ditumbuhkan dalam medium yang terdiri atas 2% proteose pepton, 0,1% ekstrak ragi, 0,1% NaCl dan 0,5 % glukosa dengan pH 7,2 ; pada suhu 29-30°C. Medium sebelumnya disterilkan dalam autoklaf pada suhu 120°C, tekanan 1 kg/cm^2 selama 15 menit. Pengamatan dilakukan tiap empat jam, terhadap sel mati (*nonmotile cells*) dan sel total (setelah dinonaktipkan dengan penambahan larutan Hayem) dengan mempergunakan hemasitometer *Neubauer improved*. Jumlah sel hidup diperoleh dari hasil pengurangan sel total dengan sel mati.

Uji aktivitas antiprotozoa

Minyak saga (EH), ekstrak etanol (EEt), residu heksana (RH) dan residu saga (RS) masing-masing ditambahkan ke dalam 75 ml medium pertumbuhan protozoa dalam labu erlenmeyer, dalam variasi konsentrasi 0,001-1 %. Sebelumnya RH dan RS dikeringkan pada 50°C selama 24 jam, kemudian dihaluskan sampai lolos 80 mesh. Minyak saga dicampur dengan emulsifier *Cremophore El* (0,01%) sehingga diperoleh larutan homogen dengan medium. Sterilisasi dilakukan pada suhu 120°C, tekanan 1 kg/cm^2 selama 15 menit. *T.pyriformis* yang berumur 24 jam (57,2 x 10^4 sel per ml) sebanyak 3 ml diinokulasikan pada medium yang mengandung ekstrak saga tersebut, kemudian perbenihan diinkubasi pada 30°C selama 96 jam. Pengamatan dilakukan terhadap jumlah sel mati dan sel total. Percobaan menggunakan rancangan acak lengkap dengan masing-masing 3x2 ulangan. Data diolah dengan analisis variansi pada taraf signifikan 5%. Persentase hambatan pertumbuhan dihitung dari rumus (K-S)/K x 100%, dimana K = jumlah sel hidup protozoa dalam medium tanpa ekstrak biji saga (kontrol) dan S = jumlah sel hidup protozoa dalam medium yang mengandung ekstrak biji saga.

Pemeriksaan fitokimia

Analisis kualitatif dilakukan terhadap ekstrak etanol (EEt) biji saga, dengan memeriksa adanya alkaloida (reagen Wagner, Dragendorf, Meyer), fenol (reagen diazo A & B), flavonoida (reagen etanol + NaOH 10%), saponin (tes kestabilan busa), tanin (reagen HCl), steroid/triterpen (reagen Liebermann Burchard) dan terpenoid (reagen vanilin + H_2SO_4) (16).

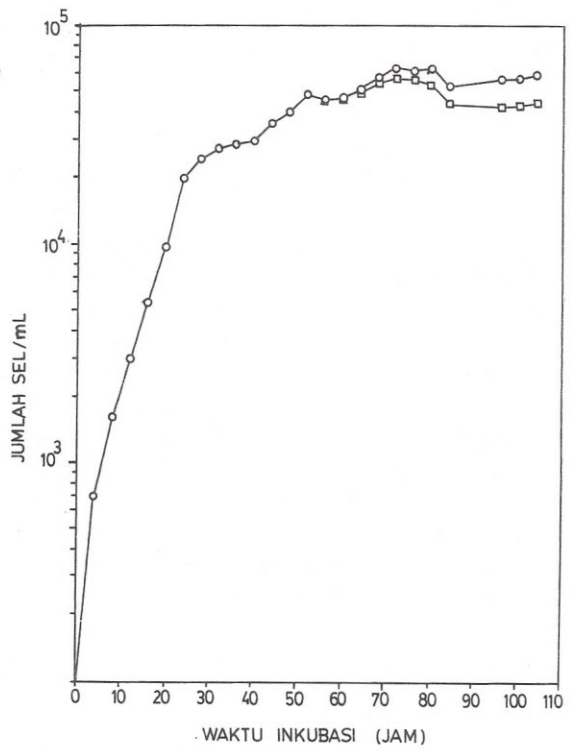
Kromatografi cair kinerja tinggi (KCKT)

Ekstrak etanol saga dianalisa dengan KCKT (Waters associates) pada kolom RP18, eluen metanol:air = 8:2 (v/v) dengan kecepatan alir 0,7 ml/menit. Sensitivitas 0,02 AUFS. Detektor yang digunakan ialah UV detektor pada panjang gelombang 313 nm. Sebelumnya contoh yang akan dianalisa dilarutkan dalam pelarut metanol:air=80:20 (v/v), dalam kadar 0,2% (b/v), kemudian disaring melalui kertas saring Millipore, cat.no. FHLP 01300, filter type FH, pore size 0,5 μm.

HASIL DAN DISKUSI

Kurva Pertumbuhan

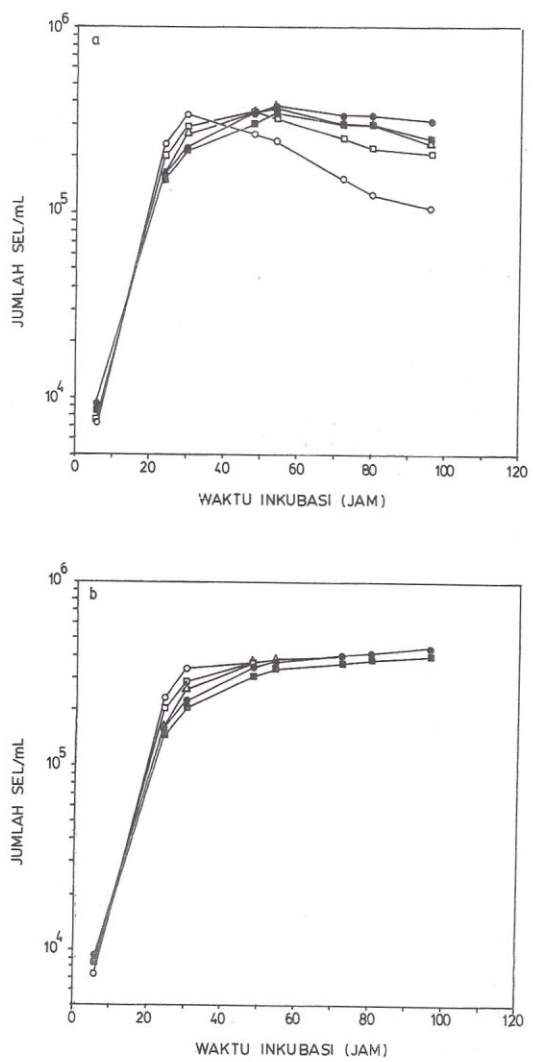
Kurva pertumbuhan *T. pyriformis* adalah seperti terlihat pada Gambar 1. Kecepatan pertumbuhan sangat pesat selama lebih kurang 24 jam terlihat dari kenaikan yang tinggi dari populasi sel protozoa (fasa logaritma). Setelah itu kecepatan pertumbuhan berkurang kemudian mulai stabil dan pada waktu inkubasi 56 jam mulai teramati adanya sel protozoa yang mati. Sehingga setelah 56 jam, pengamatan dilakukan terhadap sel total dan sel hidup, dan kurva pertumbuhan dibedakan antara kurva sel total dan sel hidup (**Gambar 1**). Dapat dikatakan bahwa fasa stasioner mulai teramati pada 56 jam waktu inkubasi. Sel protozoa yang mati terlihat sebagai sel yang tidak bergerak dan bentuk sel biasanya berubah dari lonjong menjadi bulat.



Gambar 1. Kurva pertumbuhan *T.pyriformis* GL (○) sel total, (□) sel hidup.

Pengaruh keping biji saga, residu heksan dan residu saga terhadap pertumbuhan protozoa

Pengaruh keping biji saga terhadap *T.pyriformis,* dinyatakan dalam jumlah sel total dan sel hidup selama waktu inkubasi seperti terlihat pada Gambar 2a dan 2b. Kadar 0,001 % dan 0,01 % keping biji saga dalam medium selama 56 jam inkubasi tidak menunjukkan pengaruh yang nyata (P>0,05) terhadap populasi sel protozoa. Sedangkan kadar 0,1 % dan 1 % keping biji saga menunjukkan kenaikan populasi sel total *T.pyriformis* yang nyata (P<0,05) pada waktu inkubasi 24 dan 30 jam dibandingkan dengan blanko. Setelah 30 jam, pengaruh keping biji saga terhadap populasi sel total protozoa tidak nyata (P>0,05, Gambar 2b). Jadi jika diamati dari sel total saja, tak terlihat pengaruh hambatan keping biji saga terhadap pertumbuhan *T.pyriformis;* tetapi yang teramati ialah kenaikan populasi sel seperti yang disebutkan diatas. Akan tetapi sebenarnya dalam perhitungan sel total, ikut terhitung juga sel yang mati, sehingga pengaruh biji saga akan lebih jelas jika ditinjau dari populasi sel hidup. Seperti terlihat pada Gambar 2a, selama 30 jam inkubasi, populasi sel hidup protozoa sama dengan sel total, karena tidak teramati adanya sel yang mati. Pada waktu inkubasi 48 jam mulai terlihat adanya sel yang mati. Pengaruh 0,1–1 % keping biji saga terhadap populasi sel hidup protozoa terlihat setelah 48 jam, yang merupakan hambatan terhadap pertumbuhan sel protozoa. Kadar 0,10 % menunjukkan hambatan nyata mulai 72 jam sedangkan 1 % menunjukkan hambatan nyata mulai 48 jam (P<0,05). Hambatan 1 % keping biji saga berkisar antara 23,6% (48 jam) sampai 66,1% (96 jam).



Gambar 2. Kurva pertumbuhan *T.pyriformis* GL dalam medium pertumbuhan yang mengandung keping biji saga:

(a). pertumbuhan dinyatakan dengan perubahan jumlah sel hidup di dalam medium,

(b). pertumbuhan dinyatakan dengan perubahan jumlah sel total di dalam medium.

(●) tanpa biji saga, (■) dengan biji saga 0,001 %, (Δ) dengan biji saga 0,01 %, (□) dengan bijisaga 0,1 % dan (○) dengan biji saga 1 %.

EXPERIMENTAL PROCEDURES

EXPERIMENTAL

In preparation for an experiment, the particle charge is accurately weighed and placed in the empty drum. The air table, nozzle, gas and slurry tubing are installed in their position using a clamp stand. The drum covers and subcovers are put in place. The wax for coating is accurately weighed and placed in the feed wax beaker. Air flows to the nozzle and air table are turned on and set to the desired level using the rotameters. The drive to the rollers is switched on and the drum speed set at the desired level. All thermometers and thermocouples are correctly installed, then the heating plate and two air heaters are turned on. It takes approximately one hour to preheat the air and wax to the desired temperatures.

Once the air and wax have reached the desired temperature, the wax pump is switched on at a high rate to preheat the feed line without solidifying wax in the line. Once the molten wax has reached the spray nozzle, watch on, the flow rate is reduced to the desired level.

As particles begin to be coated by the wax, the air table flow rate may need to be increased to ensure smooth flow of the particles across the air table. The base case operating conditions for each particle size are particle charge of 750 g, drum speed of 20 rpm, air nozzle flow rate 19.8 l/min, coating time is 15 minute. These conditions were used in all experiments, otherwise specified.

After the desired coating time, the experiment is shut down. The slurry pump is switched off together with the stop watch to make sure the input feed time is defined. The fluidising and atomising air are then switched off as well as all the electric powers. The stand clamp, covers, subcovers, air table and the nozzle are removed. The coated particles are collected on the pan. Particles sticking to the drum wall are removed and weighed separately. The wax sticking to the drum wall is scraped off as much as possible to calculate the wax lost. The nozzle and the tube are weighed to calculate the amount of wax left in the feed lines. The rest of wax in the glass beaker was also weighed and the net feed wax to the drum calculated by difference.

PARTICLE SIZE DISTRIBUTION

At the completion of each experiment, the entire drum contents was split into two parts. One part is used to measure the particle size distribution and fraction of agglomerates formed. The other part was used to measure the binder distribution and binder capture efficiency. The mass size distribution was measured using a set of laboratory test sieves (BSS 410/1986). Particles were placed on the sieve stack (1 to 11.2 mm) and then put on the shaker for 1 hour. Each fraction was weighed and the mass fraction calculated.

The particle size distribution was expressed as the cumulative mass distribution F(x), which is the fraction of the total mass of particle that is less than size x, where x can be expressed in terms of length [13].

AGGLOMERATE MASS FRACTION

After measurement of the particle size distribution by sieving, the agglomerated particles were collected by hand from each size fraction. The agglomerates were then weighed to calculate the total mass fraction of agglomerates formed. Note that sorting of agglomerates by hand was only necessary in size fractions in which both agglomerates and coated single particles were found.

RESULTS AND DISCUSSION

PARTICLE GROWTH MECHANISM

In this experiment using model glass beads as particle and molten wax as binder it gives a simple growth mechanism, agglomeration or layering. When binder sprayed and hit the particle, the particle surface is wetted as a liquid layer. Before drying, the liquid layer build a liquid bridge to one another. If the binder is strong enough to hold particles together, agglomerate resulted, if not, particle breaking layering resulted.

PARTICLE SIZE DISTRIBUTION

Examples of coated particle size distributions are shown in Figure 5. Smaller initial particle size gave a broader particle size distribution due to the higher proportion of agglomerates formed. For larger initial particle sizes (≥ 3 mm) the granule size distribution is much narrower. There are few agglomerates, and these are generally doublets or triplets, rather than multi particle agglomerates. Clearly, prediction of the final particle size distribution is very dependent on knowing the extent of agglomerate formation.

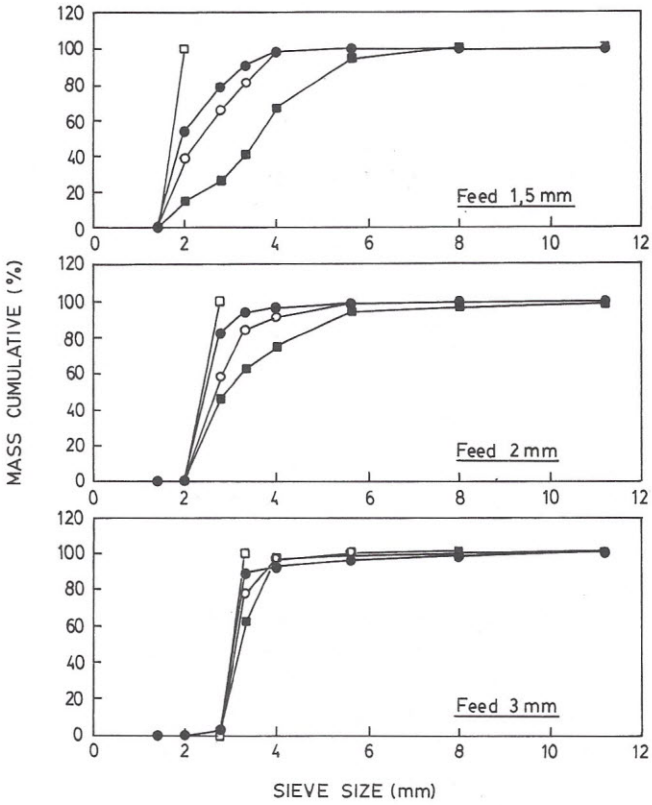


Figure 5. Particle size distribution for different spray rates, particle sizes and amount of wax added.

Wax rate (g/min)	Wax in (g)
(●) 17,6	265
(○) 31,6	315
(■) 43,4	390
(□) Initial size	

AGGLOMERATE FORMATION

Effect of particle size

Figure 6 shows the mass fraction of agglomerates formed as a function of initial particle size for 3 different spray rates of molten wax. Clearly, particle size has a significant effect on the formation of agglomerates. For particle sizes of 4 mm and above, there is virtually no agglomerate formation. As particle size is decreased below 4 mm, there is sharp increase in the level of agglomerates. At 1.5 mm particle size, 60 to 90% of the original particles are present as agglomerates, depending on spray rate conditions. These results match, at least qualitatively, the sharp transition from non-inertial to coating regime predicted by Ennis [14].

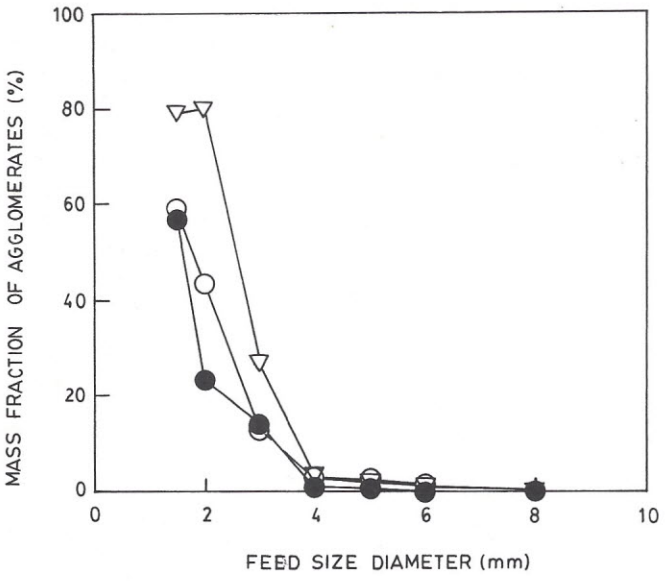


Figure 6. Agglomerate fraction for different spray rates (Wax = 265 g).
Wax rate (g/min):
(●) 17.6
(○) 31.6
(▽) 43.4

Effect of wax spray rate and amount of binder added

The effect of binder spray rate on agglomerates mass fraction is also shown in Figure 6. Increasing the spray rate increases the agglomerate formation in the critical transition (inertial) region but has no effect at large particle sizes (coating region). Alternatively, the effect of increase spray rate can be viewed as shifting to the right the curves in Figure 6. Increasing the spray rate from 17.6 to 43.4 g/min increases the initial particle size at which 50% agglomerates occur from 1.7 to 2.8 mm.

Increasing the spray rate will increase the liquid layer thickness a particle picks up on each passage through the spray zone [15]. This increases the critical Stokes number in the Ennis analysis and thus the probability of agglomerate formation. In addition, the drying time for the liquid layer will increase [15]. Particles will stay sticky for longer, increasing the chance of agglomerates forming.

In contrast, Figure 7 shows that agglomerate formation was independent of the total amount of binder added at any given spray rate, within the experimental accuracy. The amount of wax was varied between 35% and 52% of the initial mass of particles in the drum. Clearly, the thickness of the liquid layer at each pass through the spray zone, rather than the total (solid) coating thickness, is important in determining agglomerate formation.

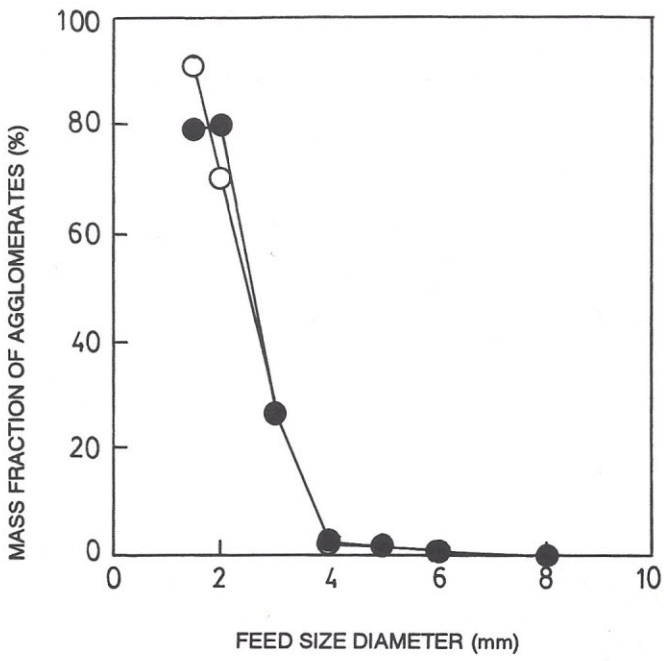


Figure 7. The fraction agglomerates for different amount of wax added at the same spray rate (43.4 g/min).
Wax in (g):
(○) 390
(●) 265

Effect of drum speed

Rotation of the drum recycles of particles within the drum and through the binder spray zone. Increase in speed increases the mass of particles recycled per unit time. As the drum speed is raised from 20 to 40 rpm, the particle recycle rate is estimated between 13.5 and 27 kg/min. For the same binder spray rate (g/min) the average amount of binder sticking on to the particles per pass through the spray zone should be proportional to drum speed. i.e. for a drum speed of 40 rpm the average layer thickness on the particles should be half that at 20 rpm. Increase in speed should reduce the average binder layer thickness [15], leading to growth by coating rather than coalescence. Increasing speed also increases the radial velocity of the drum wall from 16.2 cm/sec at 20 rpm to 32.4 cm/sec at 40 rpm. This may increase the particle relative velocity.

The experimental results show drum speed has effect on the agglomerate formation. The agglomerate mass fraction decreased slightly by increasing drum speed, as shown in Figure 8. However, the effect is small when compared to changing the spray rate. Doubling the drum speed reduces

the initial size for 50% agglomerate formation only from 2.1 mm to 1.8 mm.

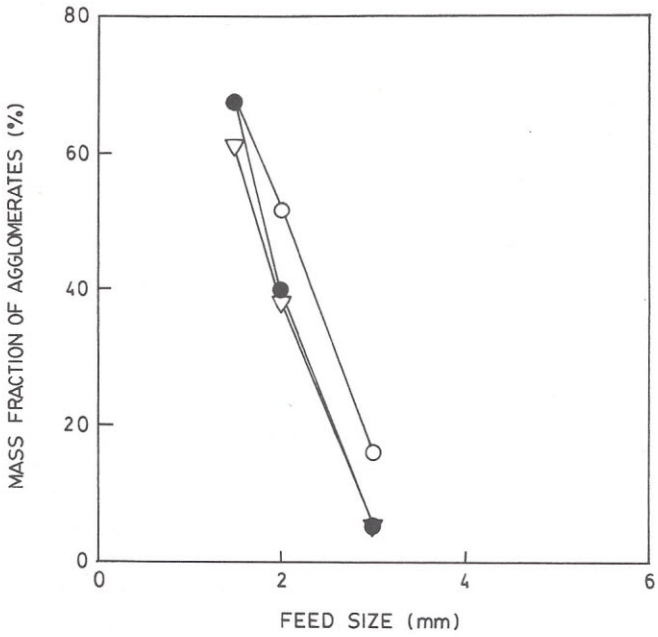


Figure 8. The fraction of agglomerates for different drum speed (spray rate of 31.4 g/min).
Drum rotation:
(○) 20 rpm
(●) 30 rpm
(▽) 40 rpm

CONCLUSION

Study of laboratory scale FDG has been done and the equipment run well. The experiments were performed to study the effect of 3 process variables on agglomerate formation. Agglomerate formation increases with decreasing particle size, increasing binder spray rate, and decreasing the drum speed.

Particle size between 3 and 4 mm is the transition from agglomerating to layering process. The spreading of particle size distribution increased with decreasing particle feed size due to the present of agglomerates. Smaller particle gave broader size distribution than bigger.

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